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Review

Cannabinoids, opioids and eating behavior: The molecular face of hedonism?

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ABSTRACT

Obesity represents nowadays one of the most devastating health threats. Published reports even project a decline in life expectancy of US citizens due to the rapidly increasing prevalence of obesity. This alarming increase is intimately linked with recent changes of environment and lifestyle in western countries. In this context, the rewarding or even addictive properties of popular food may represent one of the most serious obstacles to overcome for an effective anti-obesity therapy. Therefore, in addition to molecular networks controlling energy homeostasis, now researchers are starting to define central nervous mechanisms governing hedonic and addictive components of food intake.

A recently emerging body of data suggests that the endogenous cannabinoid and opioid systems both represent key circuits responding to the rewarding value of food. This review focuses on the role of these two systems for the homeostatic and hedonic aspects of eating behavior and includes their anatomical and functional interactions. Independent from the degree to which eating can be considered an addiction, cannabinoid and opioid receptor antagonists are promising anti-obesity drugs, since they are targeting both hedonic and homeostatic components of energy balance control.

Contents

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1. Introduction

Obesity is defined as a disease that results from the imbalance between energy intake and energy expenditure needs. Since obesity and its consequences, such as type 2 diabetes, cardiovascular diseases and cancer, are a serious health threat, it is mandatory that an effective and safe pharmacological treatment becomes available soon (Gura, 2003). The worldwide shocking increase of the obesity prevalence is strongly related to changes in environment and lifestyle (Hill et al., 2003). Decreased physical activity and overconsumption of food synergistically promote body weight increase, and the rewarding properties of popular food in western societies represent yet another obstacle for effective anti-obesity drugs (Hill et al., 2003). Thus, in many obese individuals, the homeostatic mechanisms regulating energy balance that otherwise would work perfectly are overpowered by environmental influences.

Reward and motivation have been extensively studied in the context of drug addiction, and recent evidence has suggested that addiction to food and to drugs may be based on overlapping neuronal pathways. In fact, both feeding and drug use are characterized by learned habits and preferences that are acquired and stamped by powerful rewarding reinforces (Volkow and Wise, 2005). The corticolimbic circuits which are thought to be responsible for pleasure as a natural reward associated with food or sex are implicated as well in addiction and addiction-related phenomena, such as tolerance, withdrawal and relapse (Saper et al., 2002). During the last years, a substantial body of data indicates that the endogenous cannabinoid and opioid systems play a key role both in feeding and in reward (Cota et al., 2003a; Tanda and Goldberg, 2003) and might functionally interact with each other (Manzanares et al., 1999; Tanda and Goldberg, 2003). This overview attempts to present a complete analysis of the multiple interactions between these two systems with particular focus on their importance for the regulation of food intake and on their role as potential valuable target for the treatment of obesity and eating-related disorders.

2. The endogenous cannabinoid system

The appetite inducing effects of marijuana (cannabis sativa), particularly for sweet and palatable food, have long known and are well documented in the literature (Cota et al., 2003a). Recent evidence shows now that an endogenous cannabinoid system provides the neuro-anatomical basis for the behavioral effects induced by marijuana and its derivatives, and that this system is also involved in several physiological processes relevant for memory, pain, motor activity and energy balance, among others (Di Marzo et al., 1998; Piomelli, 2003).

This endogenous system is represented by several distinct cannabinoid receptors (CB1, CB2 and likely a CB3 receptor) along with multiple endogenous ligands (named endocannabinoids) and specific biosynthetic and degradative pathways (Howlett et al., 2002). Accelerating research to unmask the multiple physiological functions of this system is ongoing.

2.1. *Cannabinoid receptors*

The cannabinoid receptors have been identified in a reverse pharmacology approach based on binding experiments of the main psychoactive component of marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC) and of synthetic cannabinoid analogues (Matsuda et al., 1990). Cannabinoid receptor type 1 (CB1), the first cannabinoid receptor cloned in 1990 (Matsuda et al., 1990), is widely distributed in the central nervous system, and it has been considered for long time as the brain-specific receptor, whereas the cannabinoid receptor type 2 (CB2), which was cloned in 1993 (Munro et al., 1993), was first believed to be exclusively expressed in peripheral tissue. However, multiple studies have recently shown CB1 to be present in the periphery (for review, see (Cota and Woods, 2005)), as well as CB2 also being localized in brain-derived immune cells (Porter and Felder, 2001). Pharmacological studies have convincingly suggested the existence of additional cannabinoid receptor subtypes, these however have yet to be

cloned and identified (Breivogel et al., 2001; Hajos et al., 2001). CB1 and CB2 are seven trans-membrane G-protein-coupled receptors (GPCRs). CB receptors use the G-inhibitory (G_i) protein subunit to induce (1) inhibition of adenylate cyclase, (2) decreased opening of voltage operated Ca^{2+} channels, (3) activation of several types of K^+ channels and (4) modulation of signal-regulated kinases (for review, see (Howlett et al., 2002)). CB1 is considered to be the most abundant GPCR in the mammalian brain, with a density comparable to that one of γ-aminobutyric acid (GABA) or glutamate receptor channels (Freund et al., 2003). Numerous anatomical, electrophysiological and pharmacological studies have provided evidence for presynaptic localization of CB1 in various brain regions and on the axon terminals of several distinct cell types (for review, see (Freund et al., 2003)). Specifically, in the hippocampus, in the neocortex and in the amygdala, CB1 is highly expressed on the axon terminals of GABAergic interneurons expressing cholecystokinin (CCK). In the basal ganglia, CB1 transcript is found in different types of neurons (those ones positive for GABA, for prodynorphin and for preproenkephalin) as well as in GABAergic interneurons containing parvalbumin. While in the hypothalamus, CB1 is localized on glutamatergic neurons (Freund et al., 2003). Due to the mostly presynaptic localization of CB1 in the CNS, the endocannabinoid system results to be able to modulate the release of several neurotransmitters, including GABA, dopamine, noradrenaline, glutamate and serotonin (Schlicker and Kathmann, 2001). Therefore, the activation of CB1 receptors seems to mediate almost all the neurochemical and behavioral effects of cannabinoids, including the development of drug tolerance, drug addiction, reward mechanisms and appetite modulation (Freund et al., 2003; Piomelli, 2003).

2.2. *Endocannabinoids*

Endocannabinoids are a group of lipid ligands including amides, esters and ethers of long-chain polyunsaturated fatty acids (Di Marzo, 1998). Due to their lipophilic nature, endocannabinoids are not stored in synaptic vesicles but synthesized upon particular stimuli by neurons following membrane depolarization and increased intracellular Ca^{2+} ion levels. After binding to CB receptors, they are rapidly inactivated by a yet undefined reuptake mechanism (Di Marzo and Matias, 2005; Piomelli, 2003). An interesting aspect of the endocannabinoids activity is the rapid induction of their synthesis, receptor activation and degradation, processes that overall regulate endocannabinoid signaling. The biochemical characteristics of the endocannabinoids, along with their particular mode of production and release, emphasize the notion that these substances might act in the CNS primarily as neuromodulators, rather than as "classical" neurotransmitters (Di Marzo et al., 1998; Piomelli, 2003). Thus, the endocannabinoids have been suggested to act "on demand", with a tightly regulated spatial and temporal selectivity and with their modulatory actions depending on "when" and "where" they are needed.

Up to now, five endocannabinoids have been identified and described:

(1) Arachidonoylethanolamide or anandamide (from the Sanskrit word 'ananda' which means 'bliss'), the first endocannabinoid in chronological order of discovery, was isolated in 1992 by Devane and coworkers from the porcine brain (Devane et al., 1992). While anandamide is the most studied CB1 ligand, its receptor affinity is only moderate (K_i values in the range of 50–100 nM) and shows the characteristics of a partial agonist, which is not fully activating the receptor (Devane et al., 1992). Apart from that, anandamide is a weak CB2 ligand (Hillard et al., 1999), and it is a functional agonist at the vanilloid receptor type 1(VR1) (Di Marzo et al., 2002). In the brain, anandamide levels widely vary according to the specific region (with the highest levels in the brainstem, hippocampus and striatum), but anandamide is produced in several peripheral tissues as well (skin, gut, liver, spleen, kidney, testis and thymus) at levels comparable to those found in the brain (pmol/g of tissue) (Bisogno et al., 1999; Sugiura et al., 2002). Substantial amounts of anandamide can even be detected and quantified in blood stream, where it seems to be partially bound to albumin (Bojesen and Hansen, 2003).

(2) 2-Arachidonoylglycerol (2-AG) has been the second endocannabinoid ligand to be discovered. While it has first been isolated from the canine gut (Mechoulam et al., 1995), 2-AG exhibits an expression pattern (in the brain as well as in peripheral tissues) similar to anandamide (Bisogno et al., 1999). In contrast to anandamide that is a partial CB1 agonist, with a promiscuous activity also at CB2 and VR1 receptors (Di Marzo et al., 2002), 2-AG is a full agonist at both CB1 (with K_i values of 1–10 μM) and CB2, and it is considered the most efficacious endocannabinoid ligand so far, being probably the true endogenous CB1 agonist (Sugiura et al., 1999, 2000). Via the CB receptors, both anandamide and 2-AG exert a variety of activities similar to those triggered by exogenous cannabinoids (Di Marzo, 1998; Di Marzo et al., 1998; Freund et al., 2003; Piomelli, 2003). Although anandamide and 2-AG are both binding CB1 and are both causing very similar effects, their biosynthetic pathways and precursors are quite different. For a more in depth review on the biosynthetic and inactivating pathways of anandamide and 2-AG, the reader should refer to (Bisogno et al., 2005; Piomelli, 2003; Sugiura et al., 2002).

(3) The third CB1 ligand was only recently isolated from the porcine brain and has been named noladin ether (Hanus et al., 2001).The first study that showed the existence of this endogenous compound reported its ability to bind CB1, causing sedation, hypothermia, intestinal immobility and mild antinociception (Hanus et al., 2001). Following investigations have been unable to report that noladin ether is produced in appreciable amounts in the mammalian brain (Oka et

al., 2003). However, the presence of a noladin-like compound has been described in the rat brain (Fezza et al., 2002).

(4) The fourth endocannabinoid, virodhamine, consists of arachidonic acid linked to ethanolamine via an ester bond (Porter et al., 2002). Virodhamine is produced by neurons in the hippocampus, the cortex and the cerebellum in similar quantities as anandamide; while in the brainstem and the striatum, its concentration is even lower than that of anandamide (Porter et al., 2002). In the periphery, especially in tissues that express CB2 (like spleen, kidney, heart and skin), the concentration of virodhamine is higher than that of anandamide. At the CB1 receptor, virodhamine is a partial agonist with in vivo antagonist activity. However, at the CB2 receptor, virodhamine acts as a full agonist (Porter et al., 2002).

(5) Finally, *N*-arachidonoyldopamine (NADA) has been the fifth endocannabinoid to be discovered (Bisogno et al., 2000). It binds CB1 (Bisogno et al., 2000), and it is the first endogenous compound identified in mammals that activates the VR1 receptors with a potency similar to the vanniloid capsaicin, the pungent extract of hot red peppers (Huang et al., 2002). Based on data from rats, it is synthesized in several distinct brain areas, with the highest concentration in the striatum, hippocampus and cerebellum (Huang et al., 2002). NADA could account for some of the CB1 and VR1 mediated effects, such as hypothermia and reduction of spontaneous physical activity. It induces hyperalgesia when administered in the peripheral nervous system and has recently been suggested to be involved in the modulation of the hippocampal plasticity (Huang et al., 2002).

The main characteristics of the endocannabinoids are briefly summarized in Table 1.

2.3. *Synthetic cannabinoid receptors ligands*

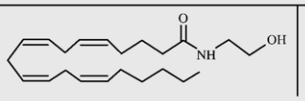
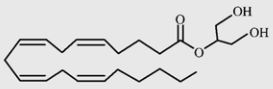
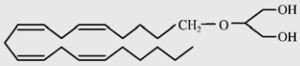
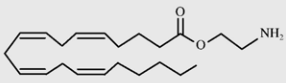
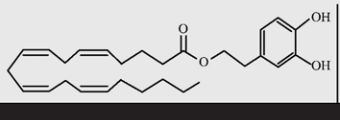
As previously mentioned, the use of the marijuana-derived Δ^9-THC and of synthetic THC analogues was instrumental for the discovery and characterization of CB1 (Matsuda et al., 1990). Among the synthetic CB1 agonists, some of them should be briefly mentioned, since they are widely used in experimental models.

HU-210 is the most potent synthetic compound belonging to the HU series synthesized at the Hebrew University in Israel and, like Δ^9-THC, is characterized by a like-3 ring structure (Howlett et al., 2002). A second group of CB1 agonists used in pharmacological studies comprises bi- and tricyclic analogs of Δ^9-THC, such as CP-55,940 (Howlett et al., 2002). Aminoalkylindols, like WIN-55,212, constitute a third group of ligands that exhibit potent agonistic activity at CB1, as well (Howlett et al., 2002).

While all the above reported compounds also show some ability to bind and activate CB2, ACEA (arachidonoyl-2′-chloroethanolamide), a structural analogue of anandamide, has been recently characterized as a very potent and extremely selective CB1 agonist without activity at CB2 (K_i values of 1.4 ± 0.3 nM) (Hillard et al., 1999).

Among the synthetic ligands showing antagonistic properties at the cannabinoid receptors, SR 141716 (Rinaldi-Carmona et al., 1994), AM251 (Gatley et al., 1996) and AM 281 (Lan et al.,

Table 1 – Characteristics of endocannabinoids

Endocannabinoid	Chemical structure	Sites of production	CB receptor binding	Peculiarities
Anandamide		Several brainareas (highest levels in brainstem, hippocampus and striatum) and peripheral organs	Partial CB1 agonist weak CB2 ligand	Detected in the blood stream bound to albumin
2-Arachidonoylglycerol (2-AG)		Several brain areas and peripheral organs (similar to anandamide)	Full agonist at both CB1 and CB2	The most efficacious endocannabinoid ligand. Produced in 170-fold greater amounts than anandamide
Noladin ether		Brain (?)	CB1 ligand (?)	Its relevance as a putative endocannabinoid still awaits confirmation
Virodhamine		Several brain areas (highest levels in hippocampus, cortex, cerebellum) and peripheral organs	Full CB2 agonist	Produced in higher amounts in the periphery than in the brain
N-arachidonoyldopamine (NADA)		Several brain areas (highest levels instriatum, hippocampus, cerebellum)	CB1 agonist	Full agonist at the vanniloid receptor 1 (with potency similar tocapsaicin)

1999) are specific for CB1. SR 141716 is of particular interest, since it is widely used in studies on the role of the cannabinoid system in food intake control (reviewed in Cota et al., 2003a). Moreover, clinical phase III studies are ongoing using SR 141716 (pharmaceutically named Rimonabant) as a possible therapy for the obesity epidemic. These studies have been recently disclosed to be encouraging (Cleland et al., 2004; Van Gaal et al., 2005).

Finally, although not acting as ligands of cannabinoid receptors, another two interesting classes of compounds are represented by inhibitors of cellular uptake of endocannabinoids, such as AM 404 (Beltramo et al., 1997), VDM-11, UCM-707 and OMDM-2 (de Lago et al., 2005), and by inhibitors of anandamide hydrolysis, such as URB532, URB597 (Kathuria et al., 2003), OL-135 (Lichtman et al., 2004) and arachidonoyl-serotonin (de Lago et al., 2005). These compounds seem to interfere with the function of the endogenous cannabinoid system trough a targeted increase in the concentration of endocannabinoids, that way possibly avoiding some of the undesirable side effects known to be associated with cannabinoid receptor agonists.

The most important synthetic and plant-derived cannabinoids compounds are briefly described in Fig. 1.

3. The opioid system

Opiates have been used as analgesics for centuries, as first reported by the Greek physician Galen. The prototype, opium, is derived from *Papaver somniferum*, commonly known as the poppy. In the 1970s, several laboratories reported that animals also synthesize opiate-like compounds, now referred to as the endogenous opioids (Bodnar and Klein, 2004). These neuroregulatory peptides are synthesized in the central nervous system and the periphery and impact both physiology and behavior. They affect a wide range of functions including nociception, memory, gastric motility, immune status, cell death, renal excretion of sodium and water, hormone secretion and food intake (Bodnar and Klein, 2004).

3.1. *Opioid receptors*

The endogenous opioids bind to a class of receptors that are members of a super-family of GPCRs (Waldhoer et al., 2004). The opioid receptors were first described in mammals in 1973 and named mu, delta and kappa. There has been a good deal of discussion related to the nomenclature of opioid receptors and their ligands (Waldhoer et al., 2004). The IUPHAR (International Union of Basic and Clinical Pharmacology) suggests that the mu, delta and kappa opioid receptors should be abbreviated as MOP-R, DOP-R and KOP-R, respectively. The delta (DOP-R) was the first opioid receptor to be cloned from a glioma hybridoma cell line (NG108-15) (Kieffer et al., 1992). This receptor has also been cloned in the rat, mouse, zebra fish, amphibian and human (Waldhoer et al., 2004). As it is true for other opioid receptors, subtypes of this receptor have been proposed based on pharmacology studies using various opioid ligands. The mu opioid receptor (MOP-R) was cloned from a variety of species, and also splice variants have been cloned (Waldhoer et al., 2004). However, these splice variants do not seem to be related to the mu subtype. The kappa opioid receptor (KOP-R) has been cloned in the zebra fish, amphibian, guinea pig, human, mouse and rat (Waldhoer et al., 2004). Once again, subreceptor types of the kappa opioid receptor have been described based on binding studies. Similar to cannabinoid receptors, mu, delta and kappa opioid receptors are capable of regulating the G_i/G_0 subunits causing inhibition of the adenylyl cyclase activity and N-type and L-type Ca^{2+} channels (Law and Loh, 1999). Activation of opioid receptors also increases phospholipase C activity, with a transient increase in intracellular Ca^{2+} levels, activation of inwardly rectifying K^+ channels and of the mitogen-activated protein kinases Erk-1 and Erk-2 (Law and Loh, 1999). One should also note that opioid receptors form dimers and heterodimers with opioid receptors as well as with other classes of GPCRs (i.e., β2-adrenergic receptor). For a more detailed description of the regulation of the opioid receptor activities, the reader should refer to (Law and Loh, 1999).

The opioid receptors are largely distributed within the central nervous system, specifically in brain structures known to modulate food intake and reward (Pert et al., 1976). Interestingly, the opioid receptor subtypes differ from each other regarding their contribution to specific opioid actions (Law and Loh, 1999). While mu opioid receptors have been shown to be involved in rewarding aspects of feeding, kappa ligands can result in dysphoria. For example, the kappa agonist Bremazocine has a potent analgesic activity, but its dysphoric effects limit its clinical use (Dortch-Carnes and Potter, 2005). It should be noted that the potency of opioid ligands on feeding behavior not only depends on the receptor subtype but also on the site of injection. More recently, Meunier et al. isolated a 17-amino acid molecule that was named nociceptin because of its ability to lower the perception threshold for painful stimuli (Meunier et al., 1997). Reinscheid and colleagues referred to nociceptin as orphanin FQ, an endogenous ligand of the "orphan" G_i/G_o-coupled opioid receptor-like 1 receptor (ORL1) (Reinscheid et al., 1995). This receptor is now termed the opioid nociceptin/orphanin FQ (N/OFQ) receptor (NOP-R). Based on receptor structure, it appears that NOP-R evolved as part of the opioid peptide receptor family (~60% homology), rather than as part of other GPCRs. The IUPHAR has suggested that the NOP-R be considered a non-opioid branch of the opioid receptors, based on ligand structure function studies.

Over the years, other receptors have been labeled as opioid-related, such as the lambda, epsilon, zeta and sigma receptors. For the most part, these receptors have not been well characterized, nor have they been cloned (Waldhoer et al., 2004).

3.2. *Endogenous opioid receptors ligands*

Three endogenous opioid ligands have been discovered and cloned during the past 30 years. β-Endorphin, enkephalin and dynorphin are derived from the prohormones proopiomelanocortin (POMC), proenkephalin and prodynorphin, respectively. These "classic" opioid peptides all share a pentapeptide sequence (YGGFM/L), containing Try-Gly-Gly-Phe as the message domain. In contrast, the mu selective agonists endomorphins (1 and 2) do not have known precursors and are structurally different from the classic

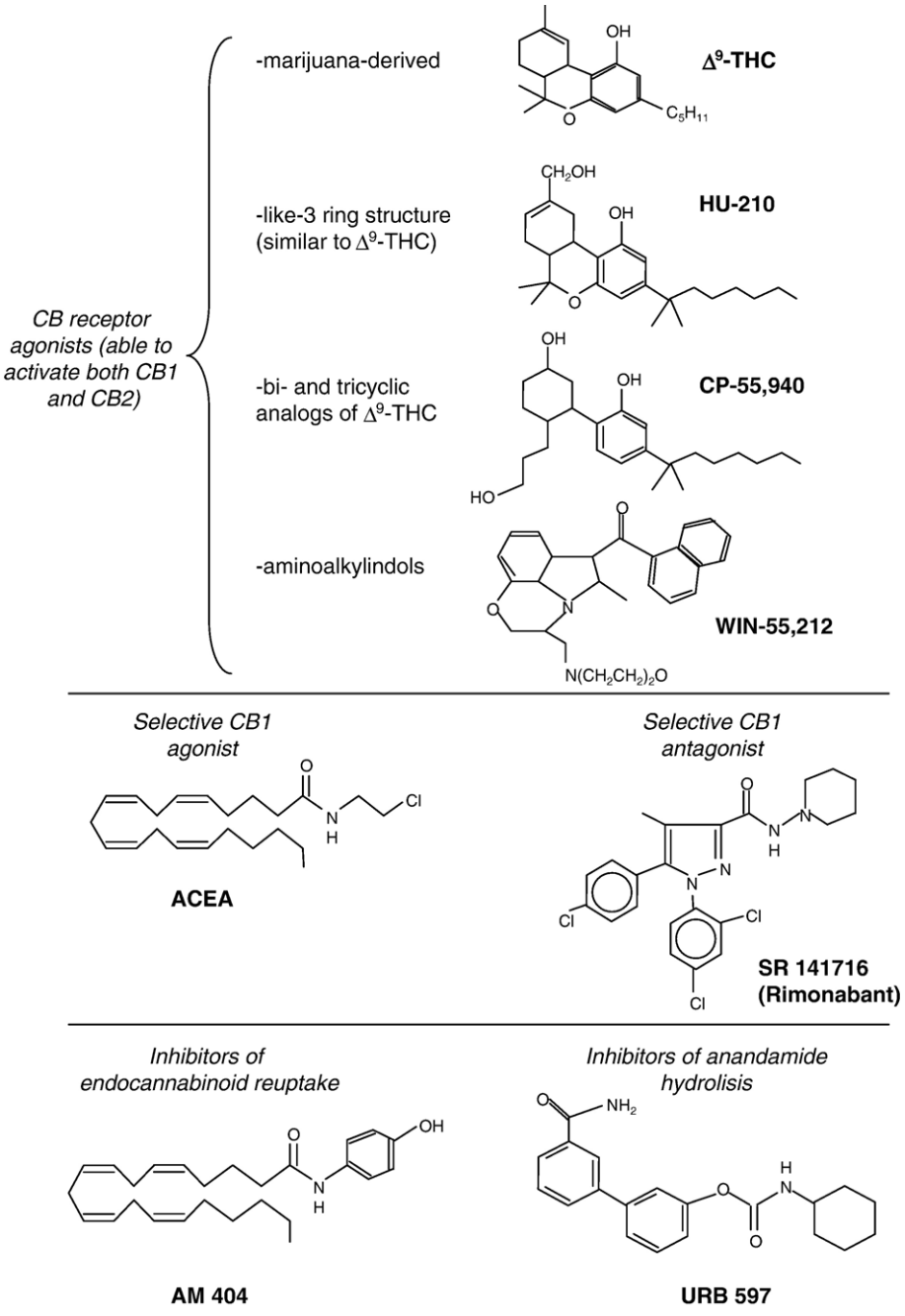


Fig. 1 – Chemical structures of the most relevant synthetic and plant-derived cannabinoid receptors ligands. The structures of representative inhibitors of endocannabinoid reuptake and of anandamide hydrolysis are also reported.

opioid peptides, being characterized by the presence of the Tyr-Pro-Phe/Trp sequence (Zadina et al., 1997). Therefore, the endogenous peptides for the mu opioid receptor include endomorphin-1, endomorphin-2, β-endorphin, β-neoendorphin and dermorphin. Especially endomorphin-1 and-2 show very high affinity (K_i = 0.36 nM and K_i = 0.69 nM, respectively) and selectivity for the mu opioid receptor, whose activation results in the regulation of gastrointestinal motility and food intake, manifestation of antinociception, and effects on the vascular systems and memory (Okada et al., 2002). Endogenous ligands for delta opioid receptor include Leu^5-enkephalin, Met^5-enkephalin, Met^5-enkephalin-Arg^6-Phe^7, Met^5-enkephalin-Arg^6-Gly^7-Leu^8, deltorphin, deltorphin I and deltorphin II. Enkephalins and their analogs are also agonists at the mu opioid receptor. Ligands for the kappa opioid receptor include dynorphin A and dynorphin B (Waldhoer et al., 2004). The structure of representative endogenous opioids is also reported in Fig. 2.

The NOP-R ligand N/OFQ does not bind to the kappa, mu or delta receptors, and opioid ligands do not serve as agonists of the NOP-R. The phenylalanine residue instead of tyrosine, characteristic for opioid peptides, present at N-terminus of the N/OFQ molecule seems to be the cause for a poor interaction between N/OFQ and the "classical" opioid

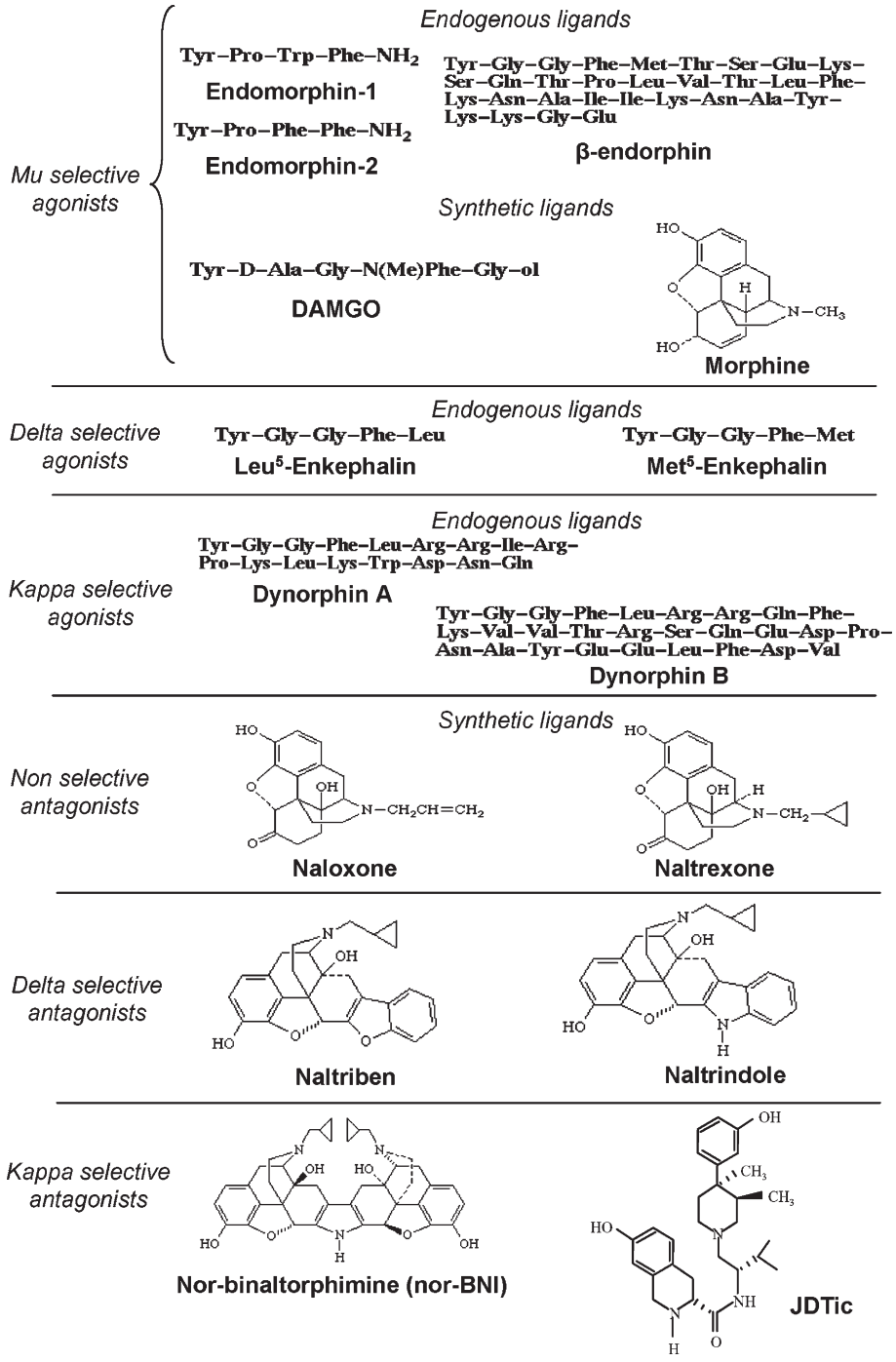


Fig. 2 – Chemical structures of the most relevant endogenous and synthetic opioid receptors ligands. Refer to the website http://www.opioid.umn.edu for a more detailed description of the structures and characteristics of the compounds.

receptors (Mouledous et al., 2000). While N/OFQ and opioids bind to different receptors, they seem to act in a similar fashion at the cellular level; that is, they activate K^+ channels and inhibit Ca^{2+} channels and adenylyl cyclase activity (Olszewski and Levine, 2004).

3.3. *Synthetic opioid receptors ligands*

There are a host of synthetic agonists and antagonists that have been synthesized for the various opioid receptors which are detailed in a website organized by the Center for Opioid Research and Design at the University of Minnesota, to which the reader should refer for a very detailed description (http://www.opioid.umn.edu). Among the opioid receptor antagonists, naloxone, naltrexone and nalmefene (non-selective ligands) should be mentioned, since these have been predominantly used for feeding studies in rodents. Moreover, these three opioid antagonists have been approved for use in man. Nalmefene and naltrexone have a longer half-life than naloxone (Dixon et al., 1986;

Licko, 1981). The most relevant synthetic opioid receptors ligands are also reported in Fig. 2.

Interestingly, several groups have suggested the existence of an endogenous anti-opioid system (Cesselin, 1995). Some endogenous anti-opioid peptides include CCK, *N*-methyl-D-aspartic acid (NMDA) receptor ligands, N/OFQ and neuropeptide FF (Cesselin, 1995). For example, CCK-2 receptor-deficient mice have more endogenous opioid present in the CNS than the wild types (Pommier et al., 2002). MK801, an NMDA receptor antagonist, blocks morphine tolerance/dependence, and chronic morphine administration alters subunits of the NMDA receptor (Zhu et al., 2003). Thus, selected neuroregulators interact with opioidergic neurons resulting in anti-opioid effects.

4. The current model of the central regulation of energy homeostasis

The involvement of various hypothalamic regions in the regulation of energy homeostasis derives from degeneration studies in the 1940s and 1950s: destruction of the hypothalamic ventromedial (VMN), paraventricular (PVN) and dorsomedial (DMN) nuclei induced hyperphagia (reviewed in Horvath and Diano, 2004). In contrast, discrete lesions placed in the lateral hypothalamus (LH) reduced food intake (reviewed in Horvath and Diano, 2004). While these approaches may be considered crude by today's standards of research, they were pivotal in pinpointing the hypothalamus as a major regulator of energy homeostasis. In fact, in retrospect to our current knowledge of specific neurotransmitter- and neuromodulator-containing hypothalamic neuronal populations (see below), these lesion studies were strikingly precise in distinguishing between subregions of the hypothalamus that house circuits that either promote or suppress feeding. The degeneration studies in conjunction with physiological observations on strains of animals that were obese (reviewed in Horvath and Diano, 2004) confirmed the proposition that humoral signals arising from the periphery may exist to inform brain sites about overall energy needs.

A fundamental breakthrough came in 1994 and 1995 when the gene encoding the adipose signal, leptin, was discovered and its product tested on leptin-deficient obese ob/ob mice (Halaas et al., 1995; Zhang et al., 1994). Subsequently, receptors for leptin were cloned (Tartaglia et al., 1995) and localized to structures of the hypothalamus (Tartaglia et al., 1995), some of which had been implicated in metabolism regulation by lesion studies. However, leptin binding and its receptors accumulated predominantly in a ventromedial hypothalamic structure, the arcuate nucleus (ARC) (Schwartz et al., 1996), an area that was not specifically suggested by the lesion studies. The appreciation of this small hypothalamic nucleus gained momentum regarding energy homeostasis when, due partially to serendipity, the melanocortin system was discovered as a player in obesity. In an earlier quest to better understand the role of various melanocortin receptors in the determination of skin color, the striking revelation was made that when melanocortin receptor 4 (MC4R) was eliminated, animals became morbidly obese (Huszar et al., 1997). Since the melanocortin system had long been known to exist in neurons of the arcuate nucleus, it was reasonable to test whether leptin exerts its effect on metabolism by the mediation of the arcuate nucleus melanocortin system. This was found to be the case (Seeley et al., 1997). Further analysis of components of the central melanocortin system delivered a plausible—although highly simplified—model, which until recently has been considered to be the primum movens of metabolism regulation. In this model, neuropeptide Y (NPY) and agouti-related protein (AgRP) expressing neurons counterbalance proopiomelanocortin (POMC) and cocaine–amphetamine regulated transcript (CART) expressing neurons in the mediobasal hypothalamus. The resulting level of balance between anabolic and catabolic drive is adjusted in communication with brainstem centers and input from the lateral hypothalamus before it is believed to be communicated to so-called second order hypothalamic neurons. Such neurons, are known to express, e.g., either thyrotrophin releasing hormone (TRH) or corticotrophin releasing hormone CRH and mainly originate in the hypothalamic paraventricular nucleus (PVN) from where they are believed to efficiently participate in regulating both, the classic endocrine axes as well as direct sympathetic nervous system outflow (for review, see (Kalra et al., 1999; Seeley and Woods, 2003)).

More recent research has determined that the adipokine leptin is not the only afferent factor to inform the CNS about the state of peripheral energy availability and metabolism. Insulin for example in addition to its multiple direct and peripheral roles in glucose homeostasis triggers satiety at leptin responsive neurons in the mediobasal hypothalamus (Seeley and Woods, 2003). This has first been shown by intracerebroventricular infusion of insulin causing hypophagia in baboons (Woods et al., 1979) and was confirmed by neuron-specific gene disruption of the insulin receptor gene causing an obese phenotype (Bruning et al., 2000).

An increasing number of gastrointestinal (GI) hormones have been also identified as important endocrine regulators of food intake, energy expenditure and body weight (reviewed in Woods, 2004).

GI hormones with satiety inducing potency are CCK, glucagon-like peptide 1(GLP-1), gastric insulin-dependent peptide (GIP), Oxyntomodulin, apolipoprotein-4 (Apo-A4), amylin and possibly PYY3-36, to just name a few. An exceptional position is assumed for the gastric peptide hormone ghrelin, since it represents the only known peripherally circulating molecule with substantial orexigenic action. Central ghrelin administration increases appetite and triggers food intake (Tschop et al., 2000). Such hunger inducing action is mainly mediated via the very same hypothalamic neurons which are targeted by both leptin and insulin to induce satiety (Cowley et al., 2003).

Due to our (although progressing) still limited insight into the neuroendocrine circuits regulating energy balance, it is yet impossible to summarize the complete picture of relevant players and essential mechanisms. However, the currently accepted model is based on the above described afferent signals informing defined neuronal circuitry in the hypothalamus and the brainstem about the peripheral energy status. In turn, these central networks are processing this information to orchestrate the appropriate efferent

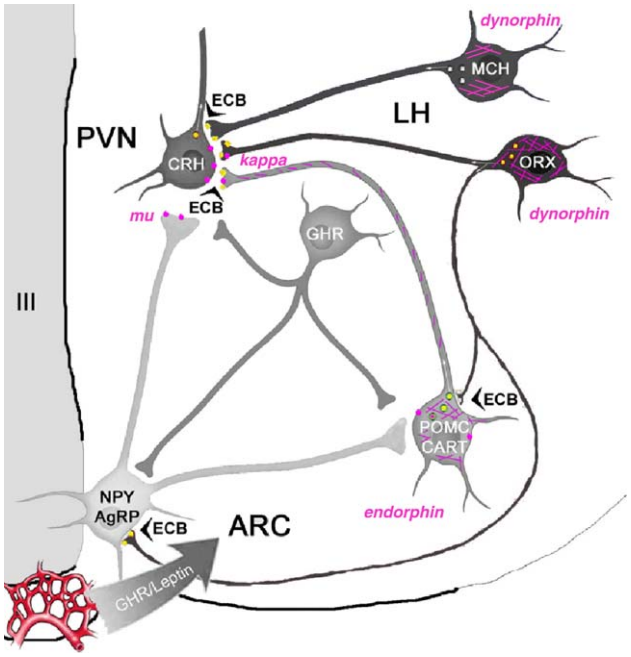


Fig. 3 – Relationship between hypothalamic peptidergic circuits, endogenous cannabinoids and opioids. The figure schematically represents expression of CB1 receptors (yellow dots) and opioid receptors (purple dots) on corticotrophin (CRH)-, orexin (ORX/Hcrt)- and proopiomelanocortin (POMC)/CART-producing neurons. CB1 is also found to be expressed on melanin concentrating hormone (MCH)-positive cells in the lateral hypothalamus (LH), while opioid receptors are present on the terminals of neuropeptide Y (NPY)/agouti-related protein (AgRP)-producing neurons in the arcuate nucleus (ARC). The purple lines identify peptidergic neurons (POMC/CART and ORX) that produce endogenous opioids (such as dynorphin and endorphin). Endocannabinoids (ECB) affect the release of neuromodulators to the synaptic cleft. Ghrelin and adiposity signals, such as leptin and insulin, influence hypothalamic peptidergic circuits via circulation. GHR: Ghrelin-producing neurons; PVN: paraventricular nucleus; III: third ventricle (adapted from Horvath and Diano, 2004).

response. Such a response aiming at energy homeostasis frequently involves a variety of systems and processes including behavioral changes, endocrine adjustments, as well as sympathetic and parasympathetic nervous system outflow modifications.

Both endocannabinoids and endogenous opioids are produced in the hypothalamus and their receptors are localized in several hypothalamic nuclei (see Fig. 3). The specific relationship of these two systems with the hypothalamic circuits in the context of food intake regulation will be further examined in the next chapters.

5. The circuits of reward and the regulation of feeding behavior

While the neuronal circuitry that ensures sufficient caloric intake by supporting the orexigenic drive is mainly represented by the hypothalamus and the brainstem (see for review (Flier, 2004; Grill and Kaplan, 2002; Kalra et al., 1999; Seeley and Woods, 2003) and previous chapter), the "liking" (pleasure/palatability) and "wanting" (appetite/incentive motivation) associated with the increased availability and variety of food are processed in the corticolimbic structures, a series of synaptically interconnected circuits linking the prefrontal cortex, the amygdala, the ventral tegmental area (VTA), the nucleus accumbens (NAc) and the ventral pallidum with the medial forebrain bundle (MFB). Such a network, through neuronal fibers connecting hindbrain and midbrain to the hypothalamic nuclei, modulates hunger and satiety (Berridge, 1996; Berthoud, 2002).

Sight, texture, smell and taste represent powerful reinforces that determine the reward value of a specific food, eventually influencing its consumption beyond homeostatic needs. A series of studies has clarified how the pleasantness of these cues is represented in the brain and how they influence feeding behavior. Data provided by neurophysiological studies in non-human primates and by functional neuroimaging studies in humans have shown that the prefrontal cortex covers an essential role in this context (see for review (Rolls, 2005)). These findings show that the visual, taste and olfactory representations of food are modulated by hunger in the orbitofrontal cortex. However, one consequence of this is that when one food is eaten to satiety, appetite for other food is still incomplete, determining enhanced eating when a variety of food is offered and therefore potentially leading to overweight and obesity (Rolls, 2005). The orbitofrontal cortex is also important as a convergence area for somatosensory inputs that are related to the texture of food (i.e., fat), processed in subcortical structures such as the nucleus of the solitary tract, thalamus and amygdala (Rolls, 2005; Saper et al., 2002).

The mesolimbic dopaminergic neurons, arising in the VTA and projecting to the NAc, are part of a brain circuit that has long been thought to play a central role in the mediation of reinforcing or rewarding action of illicit drugs as well as of food (Spanagel and Weiss, 1999). Indeed, dopamine-deficient mice die of starvation (Szczypka et al., 1999). Restoration of dopamine production within the caudate putamen of these mice restores feeding on regular chow, whereas restoration of dopamine production both in the caudate putamen and in the NAc restores preference for sucrose or for a palatable diet (Szczypka et al., 2001). Imaging studies in humans have provided evidence of dopamine involvement in the motivational properties of food intake as well as in the neurobiologic processes driving emotional eating (Volkow et al., 2002, 2003). Depending on the perceived reward value of the food stimuli, cortical and limbic areas of the human brain are activated, and an increase in extracellular dopamine in the NAc is observed (Killgore et al., 2003; Volkow et al., 2002). Similarly to what has been reported in drug-addicted subjects, striatal dopamine D2 receptor availability is significantly lower (implying lower sensitivity to reward) in obese subjects, and subjects with the lowest D2 values have the largest BMI (Wang et al., 2001). The decrease in dopamine receptor levels has been interpreted by Wang and colleagues as the "cause" for a compensatory behaviour (i.e., overeating), able to increase dopamine stimulation to a more comfortable level (Wang et al., 2001). However,

a competing explanation has been given, describing the decrease in dopamine receptors as the effect of an inherited increased activity of the dopamine pathway (Davis et al., 2004). Genetic factors also account for a role of dopamine circuits in eating disorders. Studies in the past decade have shown the association of a specific polymorphism of the dopamine D2 receptor (Allele Taq I A1) with obesity (Noble, 1994). While, the risk for the development of anorexia nervosa has been associated with the high activity allele of the catechol-O-methyltransferase gene, the enzyme involved in dopamine metabolism (Frisch et al., 2001).

Relatively little is known about the interaction between corticolimbic circuits and hypothalamus in the integrated regulation of feeding behavior. Studies performed by Kelley and colleagues have clarified that the accumbens shell, but not the core, is a critical link for the overall regulation of food intake, connecting cortical circuits and hypothalamic/brainstem circuits (Kelley, 2004). The NAc can disinhibit the neurons in the lateral hypothalamus (LHA), through the inhibition of the GABAergic striato-pallidal neurons projecting to this hypothalamic area, thus eliciting food intake (Saper et al., 2002). Conversely, feeding response induced stimulating the LHA can be prevented by blockade of striatal dopamine (Phillips and Nikaido, 1975). Interestingly, high levels of melanin concentrating hormone (MCH) receptor mRNA are expressed in the NAc, suggesting that MCH neurons, that are exclusively localized in the LHA, might have an important role in the interaction between NAc and LHA (Saito et al., 2001).

Although dopamine-mediated mechanisms have received large attention in the study of the functions processed in reward brain structures, other non-dopaminergic systems are also known to play a role. Serotonin, for example, is known to be involved in reward as well as in food intake regulation and has been targeted by numerous drugs to treat depression, obesity and related disorders (Simansky, 1996). Endogenous opioid and cannabinoid systems have also been implicated in the regulation of reward processes. Their function and role within the brain reward circuits will be examined more in detail in the following chapter.

6. Functional neuroanatomy of the cannabinoid–opioid cross-talk

An increasing number of studies indicate that cannabinoids and opioids in part use identical mechanisms to modulate physiological processes, including nociception, motor behavior, reward and appetite (Manzanares et al., 1999; Navarro et al., 2001). Consistent with this observation, distribution of CB1 receptors, opioid receptors and of their endogenous ligands has been found to be very similar within brain areas of reward circuitry (Herkenham et al., 1991; Mansour et al., 1995).

CB1 receptors are expressed on dopamine neurons in the VTA and NAc, as well as on GABAergic and glutamatergic axon terminal synapsing on dopamine neurons (reviewed in Gardner, 2005). Moreover, co-expression of CB1 with dopamine D1 and D2 receptors has recently been described, suggesting an overlap of neuronal circuits targeted by endocannabinoids and dopamine (Hermann et al., 2002). Therefore, endocannabinoids might directly modulate dopamine release (specifically in the VTA) and indirectly regulate the activity of the dopamine pathway, inhibiting GABAergic and glutamatergic neurotransmission by a presynaptic mechanism (reviewed in Gardner, 2005). Such retrograde mechanism might modulate the neuronal firing of the dopamine neurons in the VTA and NAc, regulating the reward tone. For instance, SR141716 blocks the dopamine release in the NAc induced by nicotine and alcohol (Cohen et al., 2002), corroborating the hypothesis that the endocannabinoid system has a permissive role in the release of dopamine that goes together with motivated behaviors.

Delta opioid and CB1 receptor agonists synergistically activate the mesolimbic dopaminergic system by increasing intracellular cAMP and by promoting protein kinase A-(PKA)-Cα translocation (Yao et al., 2003). Since mu opioid receptors are expressed on GABAergic terminals and opioid axons synapse on GABAergic circuit in the VTA, it has been speculated that cannabinoids might enhance dopamine neuronal activity, through the activation of the opioid circuits. Binding of opioid ligands to their receptors in the VTA inhibit GABAergic inhibitory interneurons which synapse on dopamine cells projecting to the shell of the NAc, thus increasing activity of dopaminergic neurons as measured by intracellular recordings (reviewed in Gardner, 2005). This opioid-directed disinhibition circuitry is activated by palatable food and depends on opioid signaling in the VTA (MacDonald et al., 2004).

A prototypical example of the interaction between opioid and endocannabinoid system in the modulation of motivated behaviors is given by studies that have investigated alcohol addiction. The stimulation of alcohol intake by CB1 agonists is blocked by preadministration of SR141716 or naloxone; equally, the boosting morphine's effect on alcohol intake is blocked not only by naloxone but also by SR 141716. Therefore, opioid's action is permitted by the simultaneous activation of CB1 by endocannabinoids, and, conversely, activation of opioid receptors by endogenous opioids is required for the effects of the cannabinoids (Colombo et al., 2005). Experimental evidence suggests that mesolimbic dopamine neurons are involved in mediating alcohol intake and alcohol reinforcing properties, and that both the opioid and the endocannabinoid systems affect alcohol intake trough the modulation of the mesolimbic dopamine circuit (Colombo et al., 2005).

Several studies further suggest a close relationship between the opioid and the endocannabinoid systems. For instance, the chronic administration of Δ^9-THC in rats (5 mg/kg/day, 5 days, intraperitoneal injection) can increase the synthesis of proopiomelanocortin in the hypothalamus and induce the release of endogenous opioids, like prodynorphin and proenkephalin, suggesting that chronic cannabinoids administration activate the opioid system (Corchero et al., 1997a; Corchero et al., 1997b). Whereas, when rats are chronically treated with morphine (5 mg/kg, twice a day for 5 days, subcutaneous injection), autoradiographic studies using the CB1-agonist CP-55940-stimulated (S^{35}) guanosine 5′-O-(3-thiotriphosphate) ([35S]GTPγS) binding have revealed a strong reduction (40%) in CB1/G protein coupling in the limbic area of these animals (Vigano et al., 2003). Furthermore, chronic morphine-exposed rats are characterized by a

decrease in the content of the endocannabinoid 2-AG in several brain regions, including striatum, hippocampus, limbic area and hypothalamus (Vigano et al., 2003) and limbic endocannabinoid levels result profoundly changed during different phases of behavioral sensitization to morphine (Vigano et al., 2004). In addition, both chronic alcohol and nicotine exposure modify endocannabinoid levels in the limbic forebrain (Gonzalez et al., 2002). Likewise, endogenous opioids levels result modified by the use of alcohol and other drugs of abuse (Olive et al., 2001).

Mice with a global genetic disruption of the CB1-gene ($CB1^{-/-}$) not only are unresponsive to cannabinoids but also exhibit substantially reduced sensitivity to the rewarding properties of opiate drugs as well as to naloxone-induced morphine withdrawal (Ledent et al., 1999). Furthermore, the administration of the CB1 antagonist SR141716 reduces the rewarding effects of morphine and triggers a withdrawal syndrome in opioid-dependent rats. CB1 agonists even reduce the severity of behavioral withdrawal signs in opioid-addicted rats (reviewed in Tanda and Goldberg, 2003). On the other hand, the Δ^9-THC withdrawal syndrome, for example, induced by the CB1 antagonist SR141716, is less pronounced in preproenkephalin-deficient mice (Valverde et al., 2000). Similarly, the signs and symptoms of Δ^9-THC withdrawal in mice lacking both the mu and the delta opioid receptors are attenuated, suggesting that the activity of both opioid receptor subtypes is required for the full development of a Δ^9-THC dependence (Castane et al., 2003). Moreover, the administration of 2-AG or anandamide attenuates naloxone-induced withdrawal in mice which were chronically pretreated with morphine (Vela et al., 1995; Yamaguchi et al., 2001), and, vice versa, the administration of the opioid antagonist naloxone in cannabinoid-dependent animals causes a withdrawal phenomenon (Navarro et al., 2001).

Therefore, bi-directional interactions between cannabinoid and opioid circuits seem to be mandatory for the motivational effects of drugs targeting either of the two systems (Manzanares et al., 1999; Navarro et al., 2001).

6.1. *Cannabinoids and reward circuits in the CNS: focus on the hedonic aspect of the food*

Although cannabinoids have been considered for years to lack action on the reward circuits, as briefly mentioned before, they are now known to modulate brain reward pathways, and they have been proven to interact with these brain mechanisms in a manner similar to other reward-enhancing drugs (Gardner, 2005).

Since we previously clarified that overeating might be also considered as an addiction caused by a dysregulation of brain reward substrates (see Chapter 5), it is conceivable to hypothesize that the endocannabinoid system might affect eating behavior towards the modulation of reward circuits. For example, limbic levels of dopamine as well endocannabinoids strongly correlate with craving for tasty food, indicating the co-modulatory character of their role in reward-driven eating behavior (Tanda and Goldberg, 2003). And co-expression of serotonin receptors 5-HT1B with CB1 (specifically in the striatum, in the caudate putamen and in the NAc) points to a close interaction between the serotoninergic and the endocannabinoid system (Hermann et al., 2002).

CB1 mRNA expression is down-regulated in areas of the limbic forebrain of rats fed a palatable diet (Harrold et al., 2002), effect that can be possibly explained by increased limbic levels of endocannabinoids following a diet rich in fat. Indeed, brain anandamide levels results increased after the consumption of diets containing polyunsaturated fatty acids, such as those present in condensed milk (Berger et al., 2001). Similarly, fasting increases limbic levels of anandamide and 2-AG, whereas in the hypothalamus, only 2-AG is reliably increased in this condition (Kirkham et al., 2002). Furthermore, injection of 2-AG directly in the shell of the NAc (sNAcc), a brain area which shows high expression levels of CB1, rapidly causes hyperphagia (Kirkham et al., 2002).

Regarding the ability of the endocannabinoid system to affect palatability and food choice, more than 30 years ago, anecdotal observations described the ability of marijuana (or of its main psychoactive component Δ^9-THC), not only to induce hyperphagia but also to increase the desire to consume highly palatable food in humans (reviewed in Abel, 1975). Later on, a series of more systematic studies conduced in humans confirmed that cannabinoid consumption stimulated appetite with a concomitant effect on food selection (reviewed in Cota et al., 2003a).

During the same time period, complementary studies in animal models began to shed light on some of the molecular mechanisms involved in cannabinoid-mediated hyperphagia. In 1977, Brown et al. described a dose-dependent increase in preference for palatable food and sucrose intake following low oral doses of Δ^9-THC (0.25 and 0.40 mg/kg) in rats (Brown et al., 1977). More recently, it has been described that even pre-satiated, orally Δ^9-THC-treated rats overconsumed food when compared to vehicle-treated controls, and that their food intake rates were comparable to those of fasted animals (Williams et al., 1998). Further observations of this same study not only underlined the potency of exogenous cannabinoids to induce caloric ingestion but also indicated a possible role for the endogenous cannabinoid system in feeding motivation. Indeed, when the CB1 antagonist SR 141716 (0.1, 0.5, 1.0 mg/kg, s.c.) was preadministered to prefed rats treated with the endocannabinoid anandamide (1 mg/kg, s.c.), the anandamide effect on food intake was greatly reduced, suggesting that endocannabinoids-induced hyperphagia was mediated by central CB1 receptors (Williams and Kirkham, 1999). Therefore, it was concluded that endocannabinoids might induce food overconsumption by amplifying palatability or orosensory reward (Kirkham and Williams, 2001a).

Interestingly, blockade of CB1 has a general role in the suppression of relapse to drug seeking that is independent of the type of abused drug (De Vries and Schoffelmeer, 2005). This anti-craving property is not limited to drug reinforcers but affects natural reinforcers as well. In fact, using a similar reinstatement paradigm as for heroin, cocaine and alcohol seeking, SR 141716 showed to attenuate the cue-elicited sucrose seeking following long-term extinction (De Vries et al., 2005). Moreover, SR141716 specifically reduced intake of sucrose, alcohol and sweet food in rats and marmosets (reviewed in Cota et al., 2003a; Di Marzo and Matias, 2005). However, while anorectic action of cannabinoid antagonists

more impressively decreases the ingestion of palatable food, this class of compounds to some extent also reduces the intake of bland, less tempting food (reviewed in Cota et al., 2003a; Di Marzo and Matias, 2005).

Some evidence points also to a dysregulation of the endocannabinoid system in subjects affected by eating disorders and/or obesity. Monteleone and colleagues have recently reported that plasma levels of anandamide are significantly increased in women affected by anorexia nervosa or in subjects with binge-eating disorder but not in bulimic patients (Monteleone et al., 2005). A polymorphism of the CB1 gene seems also to contribute to the susceptibility to different types (restricting or binging/purging) of anorexia nervosa (Siegfried et al., 2004). And a missense mutation of the gene encoding fatty acid amide hydrolase, the primary enzyme for the inactivation of endocannabinoids, has been associated with drug abuse (Sipe et al., 2002) as well as with overweight and obesity (Sipe et al., 2005).

6.2. *Opioids and the reward circuits in the CNS: focus on the hedonic aspect of the food*

During the past 25 years, many investigations have focused on the role of the opioid system in the rewarding aspects of feeding behavior (Kelley et al., 2002; Le Foll and Billington, 2004). Many of these studies showed a strong relationship between opioids and the "liking" component of reward using sweet tastants. For example, the opioid antagonist naloxone is known to decrease the intake of sucrose solutions much more effectively than the intake of water (Levine et al., 1982). Moreover, naloxone blocks the preference for an orange odor paired with intraoral sucrose infusion in 6-day-old rats (Shide and Blass, 1991) and blunts the increase in the development of a preference for a saccharin solution (Lynch, 1986). Also, the hedonic property of sucrose solutions, as measured by a taste reactivity test, is decreased in rats injected with naltrexone (Parker et al., 1992). Moreover, chronically injected naltrexone decreases the intake of laboratory chow plus 32% sucrose solution more effectively than the intake of chow alone (Marks-Kaufman et al., 1984). Interestingly, such a reduction in the hedonic property of sucrose following naltrexone administration has also been found in human subjects (Bertino et al., 1991). On the other side, the administration of the selective mu opioid agonist [D-Ala(2), *N*-Me-Phe(4), Gly-ol(5)]-enkephalin (DAMGO) is able to increase saccharin intake when injected into the nucleus accumbens (Zhang and Kelly, 2002). Finally, the use of genetic models has further validated the role of the opioid system in sweet taste preference. It has been described that CXBK mice, which are deficient in mu opioid receptor subtypes, prefer saccharin less than do intact mice (Yirmiya et al., 1988).

One explanation for a decrease in the hedonic property of sucrose under the influence of opioid antagonists might be that opioids are involved in taste discrimination. If an opioid antagonist blocked the ability to discriminate sweet from non-sweet, one would assume that the pleasantness of the sweet tastant would be diminished. However, O'Hare et al. found that the opioid antagonist naloxone did not alter sucrose discrimination in rats trained to discriminate a 5% or 10% sucrose solution from water using an operant procedure (O'Hare et al., 1997). Arbisi et al. found that oral naltrexone failed to affect sucrose taste discrimination in humans when compared with oral placebo (Arbisi et al., 1999).

Several laboratories have examined whether opioid receptor blockade affects the development or expression of a preference for diets as well (Le Foll and Billington, 2004). In our own studies, we have observed at several occasions that peripheral naloxone decreases intake of sweet diets more effectively than non-sweet diets. In one of these investigations, rats were given a choice between a starch- and a sucrose-rich diet for 10 days. As it has been shown previously, it became apparent that rats preferred the sucrose-rich diet. Following this, the rats were given the starch diet alone for 10 days. At the end of this period, the rats were implanted with a naltrexone or saline-filled miniosmotic pump (70 μg/h, 1.7 mg/day) for 10 days and again offered the high sucrose and high starch diet. Rats implanted with the naltrexone-filled pump ingested only about 33% of their energy from the sucrose diet, whereas the rats implanted with the saline-filled pump ingested about 77% of their energy from the sucrose diet (Levine et al., 2002).

Bodnar and Sclafani's laboratories, however, have not been able to demonstrate that opioids are involved in the development of a sweet taste preference. They evaluated whether naltrexone might alter acquisition and expression of a conditioned flavor preference in sham-fed rats given an arbitrary flavor paired with saccharin or sucrose solutions. Naltrexone decreased intake of the flavor paired with the preferred sucrose solution but failed to affect acquisition or expression of the preference (Yu et al., 1999). They also found that naltrexone decreased intake but failed to affect acquisition or expression of a preference associated with an intragastric infusion of a 16% sucrose solution (Azzara et al., 2000). Finally, they noted that naltrexone inhibited expression but not acquisition of a place preference associated with sucrose (Delamater et al., 2000). However, it should be noted that these studies, unlike the ones described above, are dependent on conditioning and learning. Since opioids affect learning (Canli et al., 1990), it is possible that conditioned taste preferences might respond differently to opioid receptor blockade than unconditioned ingestion of food or fluids.

If opioids are involved in the palatability of food and flavored solutions, then the activation level of the opioid system should respond to palatable ingesta. Indeed, the consumption of highly palatable, sweet food causes an immediate release of β-endorphin in the hypothalamus of non-deprived rats (Mercer and Holder, 1997). Similarly, palatability-induced hyperphagia determines increased hypothalamic dynorphin peptide and mRNA levels (Welch et al., 1996). Therefore, opioid levels can be modulated by the ingestion of rewarding food. Levine and Billington have suggested that a "hedonic deprivation state" might involve endogenous opioids. It has been described that when rats exposed to high palatable diet (i.e., chocolate "Ensure") are subsequently switched to chow, both POMC and dynorphin mRNA levels decrease in the arcuate nucleus (ARC) of the hypothalamus. Comparably, the restricted consumption of a palatable diet causes a decrease in striatal enkephalin gene expression,

resembling changes in opioid levels during energy deprivation (Le Foll and Billington, 2004).

Moreover, Colantuoni et al. found that when naloxone was administered to rats chronically ingesting a 25% glucose solution, the rats demonstrated behavioral changes associated with opiate withdrawal. They also noted that the chronic exposure to the glucose solution increased mu opioid receptor occupation in a variety of brain regions, including the sNAcc (Colantuoni et al., 2001).

Injections of opioids into selected brain nuclei have unveiled a role for opioids in diet preference as well as macronutrient choice. Studies have demonstrated that the infusion of DAMGO into the nucleus accumbens determines an increase both in high fat and high carbohydrate diet, when the diets are presented separately (Zhang et al., 1998). But when the diets are presented concurrently, the rats infused with DAMGO increased the intake of the high-fat diet, but not the high-carbohydrate diet, compared to vehicle-treated animals. This effect was blocked by naloxone injections directly into the nucleus accumbens (Kelley et al., 2002). Research focused on the opioid circuits in the nucleus accumbens and ventral striatum has shown a clear interaction with the GABA system. Indeed, many of the GABA neurons of these limbic areas also contain enkephalin and β-endorphin and express dense levels of mu, delta and kappa receptors (Kelley et al., 2002). Stimulation of mu receptors within the NAc (i.e., administration of DAMGO) determines increased c-Fos levels in several brain structures such as the LH and DMN, the midbrain and gustatory–visceral relay areas (Kelley et al., 2002). This suggests that the food consumption due to the activation of the accumbens opioid system recruits downstream regions that may integrate motivational, autonomic and metabolic aspects of ingestive behavior (Kelley et al., 2002). Interestingly, the efferent signals could be blocked by the administration of the $GABA_A$ agonist muscimol, therefore inhibiting the DAMGO-induced feeding effect (Kelley et al., 2002).

Moreover, very recent findings have shown that a signaling pathway exists between the VTA and the sNAcc as well, in which opioids and dopamine facilitate feeding in an interdependent manner. Indeed, food intake elicited by unilateral injections of DAMGO (2 or 5 nmol) in the VTA is dose dependently diminished by bilateral injections of the dopamine D1 receptor antagonist SCH 23390 in the sNAcc (MacDonald et al., 2004). When, vice versa, DAMGO is injected in the sNAcc, its orexigenic effect is decreased by the SCH 23390 injected in the VTA, but not by the administration of a dopamine D2 receptor antagonist (MacDonald et al., 2004). Within the sNAcc, injections of SCH 23390 inhibited the feeding response elicited by the mu agonist DAMGO, but not by a delta opioid receptor agonist [D-Ala(2), Glu(4)]-deltorphin, while the use of a D2 selective antagonist produced inconsistent effects upon either mu or delta agonist-induced feeding (Ragnauth et al., 2000). These last data, therefore, indicate that the role of dopamine receptors in mediating opioid-induced feeding within the sNAcc is dependent on the opioid receptor subtype being stimulated and on the dopamine receptor subtype being blocked (Ragnauth et al., 2000).

The central nucleus of the amygdala (CeA) represents another brain region that exerts a role in the food preference driven by the opioid system. DAMGO injection into the CeA increases food intake and also results in internalization of the mu opioid receptors present in this brain area, indicating activation of the mu opioid receptor expressing neurons (Levine et al., 2004). Moreover, when DAMGO is administered into the CeA, it increases c-Fos expression mostly in the sNAcc, suggesting one more time that the sNAcc is involved in mediating the feeding effect of DAMGO (Levine et al., 2004). Finally, when rats were injected with naltrexone in the CeA, naltrexone reduced the intake of a preferred diet more effectively than that of a non-preferred diet (Glass et al., 2000). Curiously, when naltrexone was injected in the PVN of the hypothalamus, naltrexone's anorexigenic effect was independent of diet preference, confirming that the effects on preference are mediated by the opioid pathways present in the limbic structures (Glass et al., 2000).

Finally, components of the opioid system result altered during eating disorders. Underweight anorectics have lower CSF concentrations of several peptides derived from proopiomelanocortin (POMC). When the patients regained their weight, normalization in the levels of these POMC-derived peptides have been reported (Kaye et al., 1987). Bulimic women also show reduction in the CSF levels of beta-endorphin, but not dynorphin, compared to female controls (Brewerton et al., 1992).

6.3. *Cannabinoids and the hypothalamic control of food intake*

In light of the role of the hypothalamus in the regulation of daily energy homeostasis, the localization of the endocannabinoid system in hypothalamic sites (see Fig. 3) certainly is highly suggestive for its involvement in the regulation of food intake. Among the peripheral afferent hormonal signals, leptin, ghrelin and insulin have received the most attention in terms of their role in the regulation of energy homeostasis (Horvath et al., 2001; Obici and Rossetti, 2003; O'Rahilly et al., 2003; Woods, 2004). In this context, it is noteworthy that leptin has been shown to specifically reduce hypothalamic endocannabinoid levels, without affecting their production in other brain regions (Di Marzo et al., 2001). Genetically obese, hyperphagic animal models with defective leptin signaling have elevated hypothalamic endocannabinoid levels, and the administration of the CB1 antagonist SR141716 decreases their food intake (Di Marzo et al., 2001). Moreover, injection of anandamide into the VMN of presatiated rats results in CB1-dependent hyperphagia (Jamshidi and Taylor, 2001). And the threshold level to induce feeding by electrical stimulation of the LH can be reduced by Δ^9-THC in rats (Trojniar and Wise, 1991).

CB1 mRNA has been co-localized with several hypothalamic neuropeptides, such as corticotrophin releasing hormone (CRH), cocaine amphetamine regulated transcript (CART), preproorexin and MCH (Cota et al., 2003b). It therefore seems reasonable to hypothesize that endocannabinoids may alter or be influenced by signaling flow within these hypothalamic circuits regulating food intake (Horvath, 2003). Therefore, the altered expression of some of these neuropeptides, such as CRH and CART, in the hypothalamus of $CB1^{-/-}$ mice may point to a functional relationship between these neuropeptidergic

circuits and the phenotype of this animal model in the absence of CB1 (Cota et al., 2003b). However, the decreased body weight, the reduced fat mass and the hypophagia that characterize the $CB1^{-/-}$ mice seem also to reflect the inability of potent orexigenic factors, such as neuropeptide Y (NPY) and Agouti-related protein (AgRP), to compensate for the lack of the endogenous cannabinoid action, unrevealing a crucial role for the endogenous cannabinoid system in ensuring energy homeostasis (Cota et al., 2003b). NPY is unable to increase food intake in $CB1^{-/-}$ mice (Poncelet et al., 2003). Conversely, administration of the CB1 antagonist SR 141716 to $NPY^{-/-}$ mice reduces food intake (Di Marzo et al., 2001). Therefore, while NPY is not essential for the endocannabinoid modulation of food intake, CB1 are necessary for NPY's orexigenic action and might act downstream NPY (Di Marzo and Matias, 2005).

Given the fact that glucocorticoids are among the strongest endogenous agents with orexigenic potency, it seems also intriguing that a mechanism of rapid glucocorticoid feedback inhibition of hypothalamic hormone secretion has recently been described to function via endocannabinoid release in the PVN (Di et al., 2003).

Finally, a very recent study has highlighted the existence of a functional interaction between endocannabinoids and orexins, peptides with a potential orexigenic but certainly arousal-inducing function, which are expressed in the LH. Indeed, the administration of CB1 antagonist SR 141716 can block the hyperphagia induced by orexin A and, the co-expression of CB1 with orexin 1 receptor in vitro enhances orexin signaling (Hilairet et al., 2003).

6.4. *Opioids and the hypothalamic control of food intake*

As discussed in the preview chapters, many investigations have led to the suggestion that opioids are important regulators of the rewarding aspects (liking/preference) of food intake. In this context, it is worth mentioning that both mu and kappa opioid receptors have been involved in modulating feeding of palatable food in sated animals. Whereas some studies report a more prominent role for kappa opioid receptors in the feeding response induced by fasting (Barton et al., 1996). Therefore, evidence suggests involvement of the opioid system in feeding associated with energy needs.

Early reports described that opioid receptor agonists increase, while antagonists decrease intake of high fat diets more than intake of high carbohydrate diets (reviewed in Le Foll and Billington, 2004). However, the opioid effect on macronutrient selection appears to be more complex than initially anticipated and, as previously reported, affected by the existence of diet preference (reviewed in Le Foll and Billington, 2004).

Other studies have been performed using central administration of opioid agonists and antagonists to investigate their action in discrete hypothalamic areas of the brain. When morphine is acutely injected into the VMN or in the LH, it stimulates food intake (Gulati and Ray, 1995). However, these effects appear to depend on the circadian rhythm, since they are more marked during the dark phase (Gulati and Ray, 1995). Also N/OFQ, administered directly into the VMN as well as directly into the sNAcc, has orexigenic effects (Stratford et al., 1997). Moreover, it has been shown that i.c.v. administration of N/OFQ increased c-Fos expression in the PVN and suprachiasmatic nucleus of the hypothalamus, among other brain regions (Olszewski et al., 2000).

Blockade of opioid receptors has been shown to decrease intake induced by a variety of hypothalamic orexigenic peptides, including NPY, AgRP and orexins. Injection of naltrexone into the nucleus of the solitary tract decreases intake stimulated by intra-PVN injection of NPY. However, there is little effect of naltrexone on NPY feeding when both peptides are injected into the PVN (Kotz et al., 1995). When naltrexone has been administered subcutaneously and chronically (minipumps, 70 μg/h) in fed ad libitum and food-deprived rats, it has unexpectedly increased hypothalamic ARC NPY mRNA levels, an observation that has been not explained by a difference in food intake or insulin levels (Kotz et al., 1996). Conversely, NPY has been reported to modulate opioids. Indeed, NPY stimulates β-endorphin release from the mediobasal hypothalamus in vitro and decreases POMC mRNA in the ARC of the hypothalamus in vivo (Garcia de Yebenes et al., 1995; Pau et al., 1991).

Recently, it has been described that the combined blockade of both mu and kappa opioid receptors prevents the acute orexigenic effect of centrally administered AgRP (Brugman et al., 2002). These findings suggest that the opioid system is an important downstream mediator of the potent central action of AgRP on food intake (Brugman et al., 2002).

In similar fashion, it has been shown that central opioid pathways are essential for orexin A-induced feeding. Rats were first injected with naltrexone peripherally, centrally or directly into brain areas such as the LHA or the NAc. This was followed by an injection of orexin A in the LHA. The opioid antagonist blocked orexin-induced feeding when given peripherally, ic.v. or directly into the NAc, revealing the necessity of functional opioidergic pathways involving the nucleus accumbens in mediating the orexin-induced food intake (Sweet et al., 2004). However, administration of naltrexone directly into the LHA did not influence the orexin-induced stimulation of feeding behavior (Sweet et al., 2004). Moreover, the nearly complete co-localization of dynorphin with orexin suggests that this endogenous opioid may play an important role in the functions of the orexin neurons (Chou et al., 2001). In contrast, the opioid system does not seem to be implicated in mediating the central orexigenic effect of other peptides of the LHA, such as MCH (Clegg et al., 2002).

Opioids also seem to be involved in meal maintenance by increasing the length of the meal. Smith has emphasized that meal size is a major determinant of energy intake (Smith, 1996). Naloxone does not reduce initiation of food intake but hastens the development of satiety (Kirkham and Blundell, 1984). The role of the opioid system in meal maintenance has been confirmed by many other studies in which naloxone has been shown to decrease caloric intake only after the animals have consumed a substantial amount of food (reviewed in Le Foll and Billington, 2004).

6.5. *Evidence of a cannabinoid–opioid interaction in the regulation of food intake*

Some of the more recent publications are indicating that an interaction between the opioid and the cannabinoid system

(as briefly reviewed in Chapter 6) might not only be relevant as a molecular basis for dependence but also represent the crucial key toward an understanding of the addictive component of food intake. Gallate and coworkers for example found that the enhancing effects of a CB1 agonist on the consumption of palatable beverages (such as beer) could be reversed by the opioid receptor antagonist naloxone (Gallate et al., 1999). Kirkham and colleagues have provided further support for an interaction between endocannabinoids and opioids in the regulation of feeding in rats. They described that CB1 antagonist SR141716 and opioid antagonist naloxone, used at doses which are subanorectic when administered alone (0.1, 0.5 and 1 mg/kg, s.c.), potently suppressed nutrient intake when administered together (Kirkham and Williams, 2001b). Similarly, while oral administration of nalmefene alone fails to affect food intake in mice, the combined administration of this opioid antagonist with subanorectic doses of the CB1 antagonist AM251, significantly reduced food intake (Chen et al., 2004). When higher, anorectic doses of AM251 were used, the appetite-reducing effect was further enhanced by co-administration with nalmefene and was reported in both lean and diet-induced obese mice (Chen et al., 2004). Conversely, the acute hyperphagia induced by the oral administration of Δ^9-THC in rats can be attenuated by combined subanorectic doses of the CB1 and the opioid receptor antagonists (Williams and Kirkham, 2002). However, only the work by Rowland and colleagues has clearly described the presence of a synergistic interaction between CB1 and opioid antagonists (Rowland et al., 2001). For this purpose, the authors used isobolograms to show that the combination of SR 141716 and naloxone generated a supra-additive anorectic action. No chronic studies have investigated yet whether the acute anorectic effects obtained by the combined administration of opioid and CB1 antagonists results in a greater weight loss. Finally, very recent findings have shown that the administration of naloxone in rats modulates cannabinoid receptor agonist Δ^9-THC-induced c-Fos expression in appetite-related brain areas (both compounds at 10mg/kg, i.p.). This includes regions such as the VTA, the VMN, the DMN and the CeA (Allen et al., 2003). Similarly, the administration of SR 141716 attenuated morphine-induced c-fos expression in the VTA, NAc, VMN, DMN and PVN (Singh et al., 2004). These results identify those specific brain structures as key sites for cannabinoid–opioid interaction in the regulation of food intake (see Fig. 4).

Although within the hypothalamus, the specific mechanisms involved in the endocannabinoid and opioid interaction have still to be defined, a very probable site for functional interplay between the endocannabinoid and the opioid systems is represented by the PVN. For instance, systemic administration of SR141716 is able to attenuate the overfeeding induced by the delivery of morphine within the PVN (Verty et al., 2003). Moreover, both systems are known to modulate the activity of different types of neurons located in the PVN (Di et al., 2003; Pittman et al., 1980). Concerning the limbic system, a probable substrate used by both cannabinoids and opioids is represented by the dopaminergic circuit (as discussed in Chapter 6). Also, a possible involvement of CCK may account as another mechanism for the interaction between the endocannabinoid and the opioid systems. CCK is a satiety factor highly co-expressed with CB1 in limbic structures and considered to act as an anti-opioid peptide (Marsicano and Lutz, 1999; Wiesenfeld-Hallin et al., 1999). Cannabinoids might modulate the release of CCK (Beinfeld and Connolly, 2001), therefore determining loss of the inhibition exerted by the CCK neurons on the opioid pathways.

7. Cannabinoid antagonists in the therapy of obesity

As described in the previous sections of this review, the endocannabinoid system is clearly involved in the homeostatic control of food intake and modulates food craving, thus representing a valid target for the treatment of obesity and eating-related disorders. Recent studies have evaluated the long-term efficacy of CB1 blockade on food intake and body weight in animal models, showing the ability of CB1 antagonists to interfere with peripheral and metabolic processes involved in the regulation of energy balance (reviewed in Cota and Woods, 2005). Indeed, endocannabinoids and CB1 are involved in mediating satiety signals originating in the gut, in the regulation of hepatic lipogenesis, in the adipogenesis and in the synthesis and secretion of fat-derived hormones, such as adiponectin (reviewed in Cota and Woods, 2005).

Clinical phase III trials named RIO (rimonabant in obesity) have evaluated the effects of the CB1 antagonist SR141716, pharmaceutically named rimonabant (Acomplia, Sanofi-Aventis, Paris, France), in more than 6600 overweight and obese patients with and without co-morbidities, including dyslipidemia and type 2 diabetes who were treated with the drug or placebo for 2 years. Early results from these trials have been recently disclosed.

In the RIO–Europe, after 1 year of treatment, more than 67% of patients treated with the highest oral dose of rimonabant (20 mg) achieved 5% or more weight loss, and 39% had a 10% or more weight loss (Van Gaal et al., 2005) as compared to the placebo group. The patients in the rimonabant 20 mg group achieved a reduction in waist circumference of almost 9 cm (an indirect index of the intra-abdominal fat reduction) with a significant improvement in glycemic and lipid profiles (Van Gaal et al., 2005). Interestingly, rimonabant decreased triglycerides levels and increased HDL-cholesterol independently of the effects obtained on body weight. This last outcome can be explained taking into account the ability of rimonabant to interfere with the effects of the endocannabinoids on lipid and hepatic metabolism (Osei-Hyiaman et al., 2005).

Results similar to those reported in RIO–Europe were obtained from RIO lipids, trial that evaluated the effectiveness of rimonabant in ameliorating specific co-morbidity factors associated with obesity, such as hyperlipidemia and diabetes (Cleland et al., 2004). The drug was generally well tolerated, with most frequently side effects represented by nausea, dizziness and upper respiratory tract infections (Cleland et al., 2004; Van Gaal et al., 2005).

The ability of endocannabinoids to modulate the mesolimbic dopamine circuit together with the high CB1

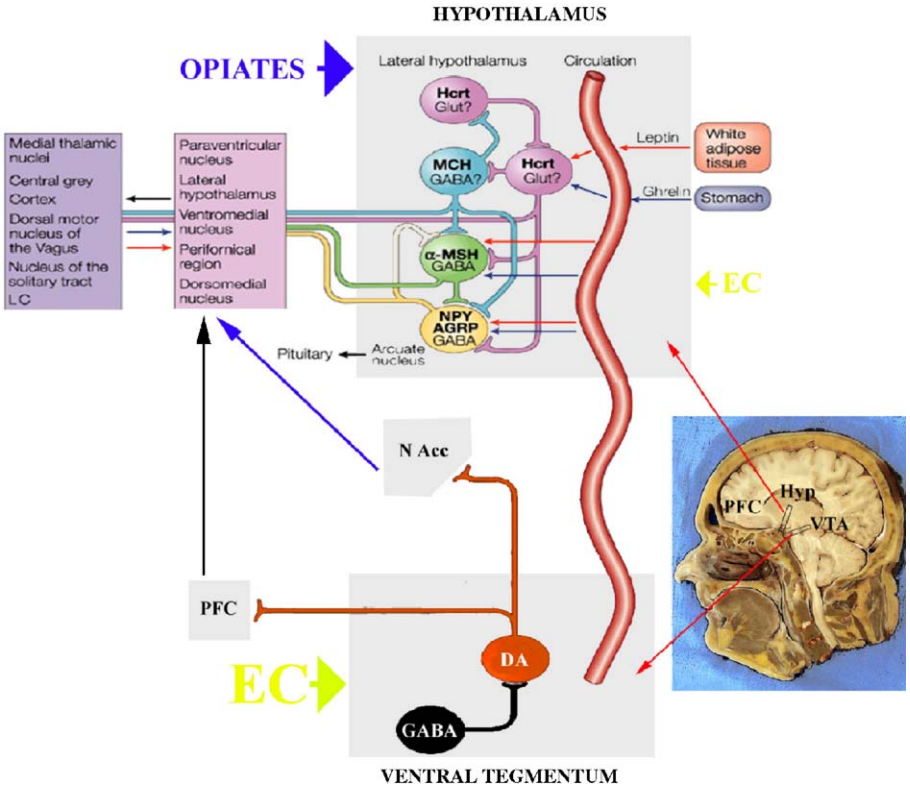


Fig. 4 – Central nervous circuits targeted by cannabinoids and opioids in the regulation of food intake. Known interactions and projections of several hypothalamic peptidergic systems, including orexin (ORX/Hcrt)- and melanin concentrating hormone (MCH)-producing neurons in the lateral hypothalamus, as well as neuropeptide Y (NPY)/agouti-related protein (AgRP)- and α-melanocyte-stimulating hormone (α-MSH)-producing neurons in the arcuate nucleus. In the hypothalamus, endocannabinoids (EC) and endogenous opioids affect the release of the abovementioned neuromodulators. In the same fashion, extrahypothalamic circuits present in reward-related brain structures, such as the ventral tegmental area (VTA), the prefrontal cortex (PFC) and the nucleus accumbens (NAcc), are affected by the actions of endocannabinoids and opioids. DA, dopamine; GABA, γ-aminobutyric acid; Glut, glutamate. Question marks indicate putative presence of neurotransmitters GABA or glutamate content (adapted from Horvath et al., 2001).

expression found in reward-related structures strongly suggests that CB1 antagonists might be also effective for the treatment of drug dependence. CB1 blockade, reducing the motivational effects of drug-related environmental stimuli, seems particularly efficacious in reducing relapse (De Vries and Schoffelmeer, 2005). Therefore, CB1 antagonists might represent a valid option for the treatment of opioid and alcohol dependence in humans (Le Foll and Goldberg, 2005). Clinical phase II trials are currently evaluating the effects of rimonabant on alcohol consumption in healthy subjects (http://www.clinicaltrials.gov). Preliminary data from the STRATUS-US trial investigating the effect of rimonabant on nicotine smokers motivated to quit are also promising. Subjects treated with rimonabant 20 mg once daily showed doubled quit rates as compared to the placebo-treated group over 10 weeks (Cleland et al., 2004). As in the RIO studies, overweight and obese smokers, who were under rimonabant treatment, lost weight and had amelioration of metabolic indices (Cleland et al., 2004).

The use of opioid receptor antagonists results to be less relevant when compared to the clinical findings obtained by administering rimonabant. A possible explanation resides in the involvement of the cannabinoid system in several aspects of the energy balance equation. The current model is that endocannabinoids act not only in the brain but also in the liver, adipocytes, gut and muscle to exert their anabolic action, and that this action results overall enhanced in obesity (reviewed in Cota and Woods, 2005; Di Marzo and Matias, 2005).

8. Opioid antagonists in the therapy of obesity

Based on the animal studies previously reported, it is conceivable to hypothesize that opioid antagonists should be useful for the therapy of obesity. There have been a number of controlled trials with intravenous naloxone, oral naltrexone and oral nalmefene on short-term food intake in healthy, normal weight, humans (reviewed in Yeomans and Gray, 2002). The number of subjects in these trials was relatively small ranging from 7 to 26, and few of the studies tested both women and men. While there was no significant effect on hunger in any of these trials, food intake decreased in all but one, and intake was reduced by 11–29%. In some of the trials, subjects reported a decrease in the pleasantness of the food (reviewed in Yeomans and Gray, 2002). It is known that

naltrexone can cause nausea in some subjects and could potentially contribute to the decreased food intake seen after naltrexone administration. About 19% of subjects in two laboratory trials reported nausea after naltrexone in contrast to 9% following placebo. However, there does not seem to be a direct correlation of nausea with the reduction of food intake in these studies.

The effect of naloxone and naltrexone on food intake in obese subjects has also been studied. The results of these studies once again demonstrate that opioid blockade decreases short-term food intake (about 33%). In four of these studies, naloxone was given intravenously, and in two of the trials, naltrexone was given orally at doses as high as 300 mg. In several of these trials, obese individuals reported a decrease in hunger. Again, there were reports of nausea after administration of the opioid antagonists.

There are conflicting results with opioid antagonists on the number of binging episodes in bulimic patients. Two controlled trials reported decreased intake, but only one of these was due to a decrease in the number of purges. Long-term food intake does not seem to be affected by naltrexone in bulimic subjects. Based on the above studies, one might assume that opioid antagonists would be useful for weight loss since they decrease short-term intake and decrease the pleasantness of palatable foods. However, there are no published trials indicating significant weight loss in obese subjects, binge eaters or in subjects with Prader–Willi syndrome following administration of opioid antagonists. These studies include naltrexone doses over a relatively wide range with doses as high as 300 mg, a dose that causes hepatotoxicity in some subjects. Moreover, no human studies have been conducted yet with selective opioid receptor antagonists. Also, naltrexone may need to be administered together with other anorectic agents, since individuals eat for many reasons and one drug effect may not be sufficient. In fact, based on the most recent animal studies where the combined administration of opioid and cannabinoid antagonists has been investigated, it may be useful to administer a CB1 antagonist together with an opioid antagonist to achieve synergistic effects on weight loss in obese humans.

9. Final synopsis and future perspectives: is eating an addiction and should be treated as such?

Food intake and energy expenditure are greatly influenced by the central nervous system. It is reasonable to suggest that the homeostatic regulation of energy metabolism, which is to support survival, is governed by hypothalamic and brainstem regions. Components of both the cannabinoid and opioid systems are present and participate in the regulation of homeostatic centers of energy metabolism, including the hypothalamus (see Fig. 3). Not considering the role of the endocannabinoid system in the peripheral metabolism (reviewed in Cota and Woods, 2005; Di Marzo and Matias, 2005), it has yet to be determined to what extent the central effect of endocannabinoids and endogenous opioids on food intake and energy expenditure are solely mediated by direct actions of these neuromodulators within the hypothalamus. In fact, experimental evidence on these systems suggests that endocannabinoids and opiates modulate food intake also via their action in the mesolimbic reward circuitry. This circuitry, in turn, feeds back to the hypothalamus, most likely predominantly at the level of the lateral hypothalamus (see Fig. 4). In parallel, the limbic pathways, particularly the ventral tegmental dopamine system as well as the efferents of the nucleus accumbens, project and carry information directly to the cortex, specifically the prefrontal cortex (see Fig. 4). This region is critical for working memory and the initiation and regulation of motivated, or eventually addictive, behaviors (see Chapter 5).

A bio-behavioral parallel exists between overeating and recognized addictive behaviors, such as abuse of alcohol, nicotine, cocaine and heroin. Brain circuits that drive addiction can be deranged with natural rewards like food as it happens with pharmacology (Volkow and Wise, 2005). For instance, highly palatable food enhances mood in humans (Davis et al., 2004) and, when consumed in excess and overtime, causes the same brain neuro-adaptations caused by drug abuse (Davis and Claridge, 1998).

The neuroanatomical and functional connection between the endogenous cannabinoid and opioid systems suggests a potentially crucial, integrative conduit at the crossways between homeostatic and reward circuits. It is essential to dissect those crossways from the multiple redundant pathways also involved in food intake regulation to possibly identify safe and efficient drug targets for the future. The ability of cannabinoids and opioids to stimulate appetite via modulation of the palatability and the preferential choice of tasty and sweet food further supports the notion that CB1 and opioid antagonists may represent a valid drug treatment for obesity, particularly for that type of disease associated with specific eating disorders, such as “sweet- and “snack-eating”.

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